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RAGE TRANSDUCES INFLAMMATION-INDUCED CHONDROCYTE HYPERTROPHY

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Aim: We examined the potential linkage of inflammation to chondrocyte hypertrophy via RAGE-dependent signaling and calgranulin expression.
Methods: We studied human OA cartilages in situ, and also stimulated primary human articular chondrocytes and normal human articular chondrocytic CH-8 cells with CXCL8, TNFa, all-trans retinoic acid (ATRA) and the calgranulin S100A11. Chondrocyte hypertrophy was defined by both type X collagen expression and size increase.
Results: Chondrocyte hypertrophic differentiation develops in OA cartilage and promotes dysregulated matrix repair and pathologic calcification that favor disease progression. The Receptor for Advanced Glycation Endproducts (RAGE) is implicated in the pathogenesis of several extraskeletal degenerative disorders linked to low-grade tissue inflammation and aging. RAGE ligands include calgranulin/S100 proteins, which have been reported to exert cytokine-like effects. The chemokine IL-8/CXCL8 stimulates chondrocyte maturation to hypertrophy, and does so dependent on both inorganic phosphate uptake and expression of TG2, an enzyme that covalently cross-links proteins by transamidation. In this study, the expression of RAGE, and far more robustly, S100A11, were upregulated in OA cartilage. In cultured chondrocytes, CXCL8 and TNFa induced S100A11 expression. In turn, recombinant S100A11 induced chondrocyte hypertrophy, an activity blocked by neutralizing RAGE antibody and soluble RAGE. S100A11 induced inorganic phosphate uptake by chondrocytes. We observed that not only S100A11- but also CXCL8- and TNFa- induced inorganic phosphate uptake were RAGE-dependent. Moreover, CXCL8- and TNFa-induced chondrocyte hypertrophy was RAGE-dependent. In contrast, ATRA-induced chondrocyte hypertrophy did not require inorganic phosphate uptake or RAGE signaling. Basal expression of the alternative calgranulin/S100 receptor CD36 was scarce in cultured chondrocytes. Significantly, transfection of either CD36, or dominant-negative RAGE, blocked the capacity of S100A11, IL-8, and TNFa to induce collagen expression. The RAGE signal transduction chain by which S100A11 induced chondrocyte hypertrophy was observed to involve MKK3 (but not MKK4), Rac1, p38 MAP Kinase, and repressed histone deacetylase 4 expression. Last, S100A11 activation of RAGE signaling is known to be optimized by S100A11 multimerization, which is catalyzed by transglutaminases (TGs). We observed that S100A11-induced chondrocyte hypertrophy was attenuated via TG2 knockdown by RNAi, but rescued in TG2-deficient chondrocytes by recombinant catalytically active TG2.
Conclusions: In this study we found that RAGE and S100A11 are upregulated in OA cartilage in situ. RAGE-specific signaling, triggered by TG2 crosslinked calgranulins including S100A11, transduces inflammation-induced chondrocyte hypertrophy. MKK3-specific signaling and inorganic phosphate uptake play central roles in RAGE-stimulated chondrocyte hypertrophy. Our results reveal novel potential target sites for therapeutic intervention to disconnect inflammation from chondrocyte dysfunction in the course of OA.

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A PEPTIDE IN THE HELICAL DOMAIN OF TYPE II COLLAGEN INDUCES COLLAGENASES AND COLLAGEN CLEAVAGE IN HUMAN OSTEOARTHRITIC CARTILAGE

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Introduction: Osteoarthritis (OA) is the result of degradation and progressive loss of extracellular matrix (ECM) in articular cartilage. Although the cause of OA is unknown, the chondrocyte mediates this process. Type II collagen is degraded by collagenases during the development of OA. MMP-1 and 13 have been shown to be present in normal and OA cartilage [1]. It has been hypothesized that the degradation products of the collagen can be potent signaling molecules and can induce a feedback mechanism in chondrocytes. The aim of this study was to investigate the effect of a peptide in the helical domain of type II collagen on collagenase expression, activation and production in human OA cartilage.
Materials and Methods: A 24 amino acid synthetic peptide (SP) lacking RGD sequence was synthesized by FMOC chemistry. Human OA chondroctyes (n=10, age range 55-89) were isolated from cartilage obtained from total knee arthoplasty. Chondrocytes were seeded at 1×10^6 cells/ml and cultured in the presence of DMEM + 10% FCS for 2 days. For simulation studies, the chondroctyes were serum starved for 12 hrs and then grown in serum-free DMEM. Gene expression for MMPs was analyzed by RT-PCR or Real-Time PCR using total RNA extracted from chondroctyes. MMP-1, MMP-13 and MMP-14 protein secreted into the medium was measured by Western blotting with rabbit anti- MMP-1, MMP-13 and MMP-13 antibodies. FAK and PKY2 activity was measured with anti-FAK and PKY2 antibodies. Anti αV and β1 integrin antibodies were used to block binding of the peptide to the respective integrins. Collagenase activity in the cultures was assayed with incubation of conditioned medium with purified pepsinized type II collagen at 25°C for 72 hrs and collagen cleavage products visualized by SDS-PAGE.
Results: The SP peptide induced both MMP-1, MMP-13 and MMP-14 mRNA in a dose dependant manner. In patients that were not expressing MMP-1 or MMP-13, the peptide always induced their transcription. FAK phosphorylation was also induced in a dose dependant manner. There was no induction of PKY2 phosphorylation by the peptide. Blocking with anti-αV and β1 integrin antibodies did not affect the production of MMP-1 and 13 induced by the peptide. Collagenase activity also increased with the incubation of the peptide in a dose-dependant manner.
Conclusions: MMP-1 and MMP-13 has been implicated in type II collagen degradation in OA cartilage. This cleavage results in production of collagen degradation fragments that can feedback and further activate MMPs. We have shown that a helical peptide of type II collagen can stimulate the production and activation of both MMP-1 and MMP-13 in OA chondrocytes. MMP-14 was also stimulated and may be responsible for the activation of the MMP-1 and MMP-13 produced by the OA chondrocytes. Further study into the signaling of this peptide and its role in MMP-1 and MMP-13 transcription, synthesis and activation could provide more targets for blocking type II collagen degradation in OA patients.

Reference

[1] Wu W, *et al.* Arthritis Rheum 2002 Aug;46(8):2087-94